sensors. The action of DHA is strikingly smaller in Slo1+ β2 (without inactivation) and in Slo1 alone, and DHA even decreases currents through Slo1+β1 (LRRC26). The stimulatory effect of DHA on Slo1+ β1 and Slo1+ β4 critically depends on two potentially interacting residues in each N terminus. The mutagenesis based on DHA-sensitive and -insensitive Slo1 channels reveals the importance of a residue in the S6 segment. The consequence of the DHA interaction with the channel involving this S6 residue is greatly amplified by the presence of β1 or β4. The β1 subunit-dependent effect of DHA underlies the blood-pressure lowering effect of the fatty acid acutely injected into anesthetized mice, and the hypotensive effect is absent in Slo1 knockout mice.

59-Subg
Powerful and Ancient Embrace of Four-Domain Voltage-Gated Channels with Calmodulin
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A most ubiquitous Ca^{2+}-sensing molecule throughout biology—calmodulin (CaM)—serves as a virtual subunit of numerous ion channels, conferring vital Ca^{2+}-dependent modulation of channel opening. Nowhere is this Ca^{2+} modulation more prevalent than in voltage-gated Ca^{2+} channels, where CaM dynamically switches among differing interactions with a proximal carboxyl tail region (CI domain), thus translating Ca^{2+} fluctuations into profound adjustments in channel opening. Using new approaches, we here uncover striking further actions of CaM. Using chemical-biological tools to step increase CaM concentration, we reveal that the binding of Ca^{2+}-free CaM (apoCaM) to channels itself imparts a striking boost in baseline open probability, with ramifications for Ca^{2+} homeostasis. Adopting a synthetic-biological approach of radical reductionism, we demonstrate that localized CaM predominates in enabling channel expression: even when the entire carboxy tail of a channel is excised, robust Ca^{2+} currents are nonetheless sustained if CaM is tethered to these 'reduced' channels. Finally, using photouncaging to produce measured step increases of Ca^{2+}, we now recognize that the CaM/CI regulatory module extends to the superfamily of Na channels, another key member of four-domain channels. Though Ca^{2+} and Na channels have generally been considered distinct, their carboxy tails exhibit tantalizing homology in the region spanning the CI domain. However, a decade of Na channel research has revealed only subtle and variable Ca^{2+} effects, with divergent mechanisms. Photouncaging of Ca^{2+} demonstrates here that the dissimilarities in Na channels are only apparent, and that function and mechanism are fundamentally conserved to an astonishing degree across Ca^{2+} and Na channels. Given the common heritage of these channels dating to the early days of eukaryotes, the present results link modern-day CI elements to a legitimately primeval modulatory design, and cast CaM as a partner in ion-channel regulation throughout much of living history.

60-Subg
Sodium Channel β1 Subunits: Overachievers of the Ion Channel Family
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Voltage gated Na^+ channels in mammals contain a pore-forming alpha subunit and one or more beta subunits. There are five mammalian beta subunits in total: beta1, beta1B, beta2, beta3, and beta4, encoded by four genes: *SCN1B-SCN4B*. With the exception of the *SCN1B* splice variant, beta1B, the subunits are type I topology transmembrane proteins. In contrast, beta1B lacks a transmembrane domain and is a secreted protein. A growing body of work shows that VGSC beta subunits are multifunctional. While they do not form the ion channel pore, beta subunits alter gating, voltage-dependence, and kinetics of VGSC alpha subunits and thus regulate cellular excitability *in vivo*. In addition to their roles in channel modulation, beta subunits are members of the immunoglobulin superfamily of cell adhesion molecules and regulate cell adhesion and migration. Beta subunits are also substrates for sequential proteolytic cleavage by secretases. An example of the multifunctional nature of beta subunits is beta1, encoded by *SCN1B*, that plays a critical role in neuronal migration and pathfinding during brain development, and whose function is dependent on Na^+ current and gamma-secretase activity. Functional deletion of *SCN1B* results in Dravet Syndrome, a severe and intractable pediatric epileptic encephalopathy. Beta subunits are emerging as key players in a wide variety of pathophysiologies, including epilepsy, cardiac arrhythmia, multiple sclerosis, Huntington's disease, neuropsychiatric disorders, neuropathic and inflammatory pain, and cancer. Beta subunits mediate multiple signaling pathways on different timescales, regulating electrical excitability, adhesion, migration, pathfinding, and transcription. Importantly, some beta subunit functions may operate independent of alpha subunits. Thus, beta subunits perform critical roles during development and disease. As such, they may prove useful in disease diagnosis and therapy.

61-Subg
TRIP(8B)Ing up and Down HCN Channel Gating and Trafficking
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The proper neuronal functioning of ion channels depends on their correct targeting to distinct polarized neuronal compartments, where the channels often mediate highly specific functions. HCN1 channels, which underlie the hyperpolarization-activated cation current (Ih) in many types of neurons, are targeted to the distal apical dendrites of hippocampal CA1 pyramidal neurons, where they regulate the integration of synaptic inputs and control excitability. Results from our laboratory and others indicate that the cytoplasmic protein TRIP8b is the major auxiliary subunit of HCN1 channels in the brain, where it plays an important role in regulating HCN1 function, expression and localization. TRIP8b undergoes extensive alternative splicing at its N-terminus, with at least 10 splice variants detected in brain. All splice variants interact strongly with the C-terminus of all four HCN channel isoforms (HCN1-4) at two different interaction surfaces. Whereas all TRIP8b isoforms inhibit channel gating by antagonizing the normal action of cAMP to facilitate opening, the various isoforms have distinct effects on channel trafficking. We identified two splice isoforms with opposing actions on HCN1 surface expression and distinct subcellular locales that are critical for HCN1 dendritic targeting. Our more recent results have identified the structural and functional bases for many of the regulatory actions of TRIP8b.

Subgroup: Motility

62-Subg
Movement of Signaling Receptors Inside Primary Cilia
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The primary cilium is a signaling organelle with a distinct complement of membrane and soluble proteins. How specific proteins are concentrated within cilia while others remain excluded is a major unanswered question. Recent work has uncovered a diffusion barrier for membrane proteins at the base of the cilium that functionally separates the topologically continuous plasma and ciliary membranes. Using a newly develop in vitro system for trafficking to cilia, we now demonstrate the existence a size-dependent permeability barrier for soluble proteins that allows the passive entry of proteins smaller than 75 kDa and forces large proteins to utilize active transport for entry into cilia. Interestingly, the ciliary permeability barrier is mechanistically unique and does not share features with the nuclear pore complex or the axonal diffusion barrier. Beyond ciliary entry, our in vitro system has enabled a study of soluble diffusion inside cilia which reveals that diffusion alone is sufficient for a rapid exploration of the ciliary space in the absence of active transport.
To further dissect the respective contributions of diffusion and active transport to the exploration of the ciliary space, we established a system for single molecule imaging of signaling receptors inside cilia. Here, we find that diffusion is the major driver of membrane proteins movement along primary cilia and that signaling receptors spend less than 25% of time undergoing motor-driven transport. Perturbation of either diffusion or active transport shows that cargo movements can be uncoupled from movements of the intraflagellar transport (IFT) machinery, and that diffusion is sufficient for membrane proteins to explore the ciliary surface. Taken together, our results indicate that signaling within cilia need not be entirely reliant on active transport and poses the question of the role of active transport in transducing signals through the primary cilium.

63-Subg
Probing Forces on Newly Generated Spindle Microtubule Minus-Ends
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The mitotic spindle is a dynamic self-organizing machine that coordinates cell division and preserves genomic stability. The ability to focus microtubule minus-ends into poles is crucial to spindle structure and function. However, our understanding of pole-focusing forces has been limited by the challenges of labeling and imaging microtubule minus-ends in established spindles. Here, we used laser ablation to sever kinetochore-fiber microtubules in mammalian cells and probe how the cell detects and organizes newly generated microtubule minus-ends. Within a few seconds of ablation, the cell recognizes

new minus-ends and begins pulling them poleward. These pole-focusing forces exist throughout metaphase and anaphase and can move chromosomes rapidly, dominating other spindle forces. Opposing forces on chromosomes from the other half-spindle are able to slow, though not stop, the pole-focusing response, as indicated by faster pole-focusing speeds in monopolar spindles and during anaphase than in metaphase bipolar spindles. Together, our data indicate that microtubule minus-end focusing forces operate broadly and rapidly and are of similar magnitude to other spindle forces. These pole-focusing forces are thus well-suited to robustly maintain spindle structural integrity despite rapid turnover of spindle components and mechanical challenges.

64-Subg
Structural, Mechanical, and Biochemical Insights into the Mechanism of Myosin Force Sensing
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Myosin-Is are widely expressed molecular motors that comprise the second largest myosin family in vertebrates with eight isoforms. Myosin-Is participate in a host of cellular processes including vesicular trafficking, membrane dynamics, and nuclear transcription. The question thus arises, how can these motors with similar biochemical properties give rise to such diversity of function? The answer appears to lie in the biochemical and mechanical diversity of the isoforms. Our biophysical studies demonstrate that Myo1b is exquisitely sensitive to tension, where forces >0.5 pN cause the motor to transform from a low duty ratio motor with attachment lifetimes <1 s to a high duty ratio motor with attachment lifetimes >50 s. Our studies also reveal that the isoform Myo1c has a very different response to force despite its similar unloaded kinetics to Myo1b. Myo1c is far less sensitive to force than Myo1b, enabling it to power motility over a range of forces, consistent with it serving a role as a transporter rather than as a tension-sensitive anchor. To better understand the molecular basis for these differences in force sensing, we have determined the crystal structure of Myo1b's motor domain and first IQ-motif with bound calmodulin. The structure reveals novel interactions between the light-chain binding domain and the N-terminus of the motor domain, a region that shows substantial sequence variability among myosin-I isoforms. We propose that these interactions facilitate communication between the lever arm and the ATP binding site, modulating the chemomechanical properties of the motor. Supported by the NIH (GM057247).

65-Subg
A Structural Model of the Kinesin-5 Mechanochemical Cycle
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Kinesin motor domains drive diverse microtubule-based, ATP-dependent activities but the molecular adaptations that specify these diverse functions are poorly understood. Kinesin-5s are essential mitotic motors and their inhibition with specific small molecules blocks cell division. Using cryo-electron microscopy and subnanometer resolution structure determination, we have visualised conformations of microtubule-bound human kinesin-5 motor domain at successive steps in its ATPase cycle. In the ATP-like state, the kinesin-5 neck-linker is directed towards the microtubule plus-end, consistent with its role in directional force generation. As ATP hydrolysis proceeds, nucleotide-dependent conformational changes in the active site are allosterically propagated into rotations of the motor domain, uncurling of the drug-binding loop5 and discrete, ratchet-like displacements of the neck linker that contribute to motor stepping. The motor N-terminus also undergoes large reorientations that indicate its role in controlling kinesin-5 neck-linker conformation throughout the motor's ATPase cycle. A kinesin-5 mutant lacking this N-terminus is enzymatically active, but ATP-dependent neck linker movement and motility is defective, although not totally ablated. Our data demonstrate that, while the motor N-terminus plays a kinetic role in controlling efficient neck-linker movement, the kinesin-5 neck-linker has intrinsic biophysical properties that enable it undergo nucleotide-dependent ratchet-like movements that have presumably evolved according to specific functional requirements.

66-Subg
From Extensile Microtubules Bundles to Synthetic Cilia and Self-Mixing Active Gels
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In presence of a macromolecular crowding agent filamentous microtubules spontaneously assemble into elongated bundles. Kinesin clusters can simultaneously bind to multiple filaments within such a bundle and thus power relative sliding of the constituent filaments and the overall bundle extension. Starting with such extensile bundles it is possible to hierarchically assemble diverse biologically inspired materials including spontaneously beating synthetic cilia, self-mixing and self-flowing active gels, motile emulsion droplets as well as deformable vesicles. However, little is known about the mechanical properties of isolated active extensile bundles that are the essential structural motif of these diverse materials. We describe an experimental technique that allows us to systematically assemble filamentous bundles with predetermined number of filaments and quantify its ability to generate extensile force.

67-Subg
Tug-of-War: Mechanical Coordination of Molecular Motors
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Molecular motors often perform their functions in small teams rather than as individual molecules. An important issue for understanding the function of motor teams is how the motors are coordinated. It has become clear in recent years that mechanical interactions between motors play an important role in such processes, as motors exert forces on each other. I will discuss two cases, where experimental evidence has recently been obtains for the presence of such mechanical interactions: bidirectional cargo transport by cytoskeletal motors of opposite polarity (e.g. kinesins and dyneins) and the twitching motility of bacteria on surfaces powered by pilus motors. Stochastic tug-of-war models explain how fast bidirectional motion (or persistent motion in random direction) is obtained despite the presence of opposing forces via an instability caused by the forced unbinding of motors. It will be shown that the mechanical interactions alone are sufficient to account for the experimentally observed dynamics.

68-Subg
Mechanisms of Dynein-Driven Microtubule Sliding and Cargo Transport
Ronald Vale[1,2], Hui-Chun Cheng[1], Gira Bhabha[1], Richard McKenney[1], Marvin Tanenbaum[1], Courtney Schroeder[1].
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Dynein is a minus-end-directed microtubule motor protein that powers the beating of cilia and flagella and transports a wide variety of cargoes within the cytoplasm of eukaryotic cells. Dynein is not evolutionarily related to the cytoskeletal motor proteins kinesin and myosin, but instead is a member of the AAA+ superfamily. Unlike most AAA+ ATPases that self-assemble into homo-hexameric rings, dynein has six distinct AAA+ domains that are concatenated within a single, large polypeptide chain; extending from one of the AAA+ domains is a long, anti-parallel coiled-coil extension (called the stalk) that binds to microtubules. We have studied the mechanism of dynein motility using a combination of structural and single molecule studies. I will discuss how the two motor domains of dynein can act to slide apart anti-parallel microtubules, an activity that may be important for dynein's actions in organizing the mitotic spindle during mitosis. I also will discuss how dynein's enzymatic cycle might be regulated for normal cargo transport.

Subgroup: Exocytosis & Endocytosis

69-Subg
Exocytotic Fusion Pore Intermediates of Dense-Core Vesicles
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Regulated exocytosis is a multistage process involving a merger between the vesicle and the plasma membranes, leading to the formation of a fusion pore, a channel, through which secretions are released from the vesicle to the cell exterior. A stimulus may influence the pore by either dilating it completely (full-fusion exocytosis) or mediating a reversible closure (transient exocytosis). In neurons, these transitions are short-lived and not accessible for